An Electrocatalytic Assay for Mutational Analysis Based on Charge Transfer through DNA Thin Films.

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DNA sensors capable of detecting mispaired nucleic acid bases are required for routine screening of genetic mutation and disease. We have been working on a strategy to detect these mismateches based on charge transport through double-stranded DNA [1]. Gold electrodes modified with pre-assembled DNA duplexes are used to monitor the electrocatalytic signal of methylene blue, a redox-active DNA intercalator, coupled to ferricyanide (Figure 1). The presence of mismatched DNA bases substantially attenuates the electrocatalytic signal. As illustrated in Figure 2, all of the possible DNA single-base mismatches can be detected using chronocoulometric analysis.

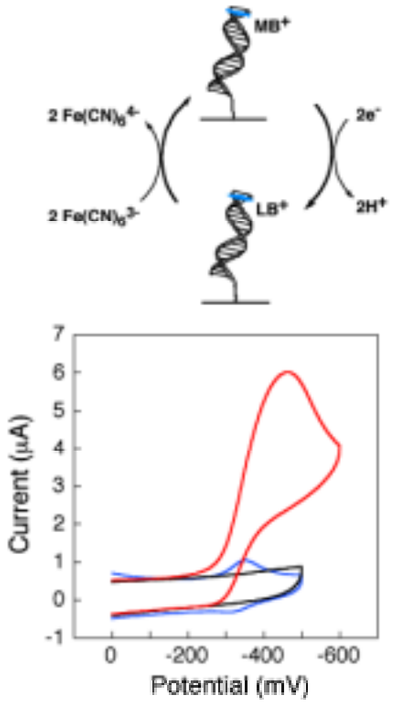

Figure 1. Electrocatalytic reduction of ferricyanide.

Natural lesions to the DNA base stack also attenuate charge transfer through these DNA films. As shown in Figure 3, the DNA products of several natural biological reactions (including oxidative damage [*e.g.*, 8-oxoA], depurination [an abasic site], and hydroxyl-radical addition [*e.g.*, 5,6-dihydrothymine]) diminish the efficiency of the electrocatalytic signal. In all cases, the magnitude of the effect correlates roughly with the thermodynamic stability of the lesion, with the least stable mismatches effecting the most pronounced attenuation.

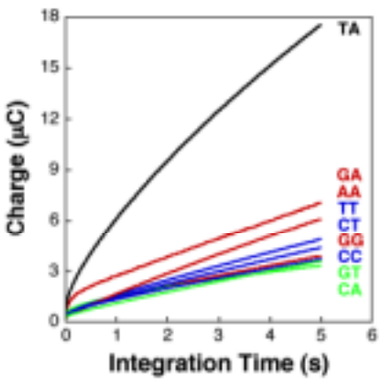

Figure 2. Electrochemical detection of DNA mismatches.

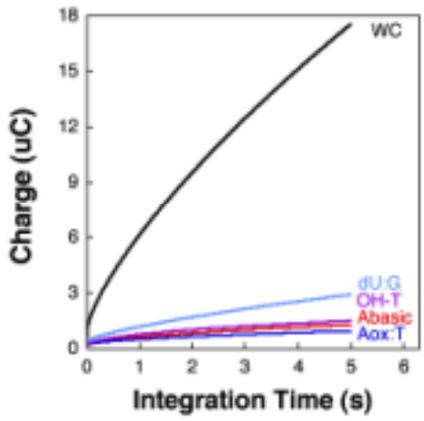

Figure 3. Electrochemical detection of DNA base lesions.

To investigate the potential usefulness of this approach for mutational analysis, sequences from the human p53 gene possessing single-base mismatches were investigated. The p53 tumor suppressor gene encodes a multifunctional transcription factor that plays a key role in the prevention of cancer; several mutational “hot spots” (codons 175, 245, 248, 249, and 273) are especially prevalent in malignant tumors. Mutations in two of these hot spots, at codons 248 and 249, were examined by electrocatalysis (Figure 4). In both cases, the mutated sequences were readily distinguishable from the native duplexes.

Reference:

[1] Boon, E. M.; Ceres, D. M.; Drummond, T. G.; Hill, M. G.; Barton, J. K. “Mutation Detection by Electrocatalysis at DNA-Modified Electrodes.” *Nature Biotech.* **2000**, *18*, 1096.